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Soft-tissue wound healing following periodontal surgery and Emdogain® application

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Abstract

Objectives: The aim of the present study was to examine, by clinical means and as patient perception of post-operative events, the effect of Emdogain® (enamel matrix derivative (EMD)) on the healing of soft-tissue wounds following periodontal surgery in comparison to flap surgeries without application of EMD.

Material and Methods: For this study, 22 subjects (nine female and 13 male) who were scheduled for periodontal flap surgery were selected. The mean age for all patients was 49.9 (SD 8.7) years (range, 32–67). Six women and five men were assigned to the test group and three women and eight men were assigned to the control group. All subjects were scheduled, after re-evaluation of the periodontal status 8 weeks after initial treatment, to undergo surgery of the soft tissues, because of existing of probing pocket depth (5 mm or more), on at least three teeth. At random, 11 patients were assigned to control treatment and 11 patients were placed in the test group. All underwent one periodontal flap surgery for the purpose of this study. A modified Widman flap was performed. Patients in the test group received an application of EMD underneath the mucoperiostal flaps and onto the exposed root surfaces. Clinical measurements were taken at four different points in time, at the time of surgery, 1, 4 and 8 weeks after surgery. All subjects filled out a questionnaire every day for the first 7 days following surgery to evaluate post-operative complaints.

Results: Of all parameters evaluated none showed a significant difference between the control and EMD groups, except for gingival swelling at the 1-week assessment, where the EMD group exhibited a higher swelling score. The questionnaire revealed that complaints of oozing of blood from the wound was twice more prevalent 1 day post-surgery in the control ($n = 6$) as in the EMD group ($n = 3$).

Conclusion: This study shows that the early woundhealing of periodontal flap-surgeries in those sites treated with Emdogain® is not different from control sites.

Key words: Emdogain®; flap surgery; periodontitis; wound healing

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Periodontitis is an infectious disease affecting the supporting tissues of the teeth. The aim of conventional periodontal therapy is to stop and possibly reverse the loss of periodontal attachment resulting from this disease. Supra- and subgingival debridement focuses on removal of bacterial plaque from teeth and periodontal pockets. Treatment usually leads to reduction of gingival inflammation, pocket reduction and recession of soft tissues.

An ideal goal of periodontal treatment would be the regeneration of tissue from the periodontal ligament. Several products, augmenting tissue regeneration, have been manufactured and have been tried in clinical practice with differing results. One of the more recently developed products is Emdogain® (Biora, Malmö, Sweden), This consists of a gel containing hydrophobic enamel matrix proteins extracted from porcine-developing embryonic enamel. Enamel matrix derivative (EMD), applied to the root surface in conjunction with surgical periodontal therapy, may promote periodontal regeneration as demonstrated in both animal experiments and clinical studies (Hammarström et al. 1997, Heijl et al. 1997, Heden et al. 1999, Froum et al. 2001).

Although EMD has been shown in numerous reports to improve clinical parameters and to mediate a regenerative healing response, the mechanism by

which EMD exerts its influence on cells has only recently been evaluated. An in vitro investigation of the effects of EMD on the behavior of human periodontal ligament cells (PDL) and gingival fibroblasts indicated that both cell types release significantly higher levels of transforming growth factor β1 under the influence of EMD than in control cultures (Van der Pauw et al. 2000, 2002, Lyngstadaas et al. 2001). Another in vitro study demonstrated that PDL cell wound fill rates are increased significantly compared to those of gingival fibroblasts when EMD is added to a medium containing both cell types. EMD not only had an effect on the migration of periodontal ligament cells but also on gingival fibroblasts (Hoang et al. 2000). Taken together, these observations indicate that EMD, in addition of being a means for periodontal regeneration, may influence soft-tissue healing.

A recent study evaluated by clinical means the effect of enamel matrix proteins on the healing of a soft-tissue wound produced by periodontal pocket instrumentation (Wennström & Lindhe 2002). All experimental sites were scaled and root planed, and the soft-tissue wall of the pocket was curetted to remove the pocket epithelium and adjacent granulation tissue. The site was carefully irrigated with saline. When the bleeding from the pocket had ceased, a 24% EDTA gel was applied in the site and retained for 2 min. This was followed by careful irrigation with saline. Left and right jaw quadrants were then randomized to subgingival application of EMD or vehicle control. All sites were re-examined after 1, 2 and 3 weeks. The results indicated that Emdogain® topically applied in instrumented pockets enhances the early healing of periodontal soft-tissue wounds. Patients also reported significantly less post-treatment discomfort at sites subjected to EMD application.

The aim of the present study was to examine, by clinical means and as patient perception of post-operative events, the effect of EMD on the healing of soft-tissue wounds following periodontal surgery in comparison to flap surgeries without application of EMD.

Material and Methods

Sample

For this study, 22 subjects (nine female and 13 male) who were scheduled for periodontal flap surgery were selected. All the subjects had been referred to the Academic Center for Dentistry in Amsterdam (ACTA), the Netherlands, for treatment of periodontal disease. They were diagnosed as suffering from periodontitis. Supra- and subgingival debridement had been performed by undergraduate dental students taking part in an expanded perioprogram. The initial periodontal treatment was performed under local anesthesia in four 45–60 min sessions. Instrumentation was carried out until no supragingival plaque or calculus was visible and all pathologically exposed subgingival root surfaces felt hard and smooth using a fine explorer. If a dental student was not able to reach this treatment goal, the treatment was completed by the instructor.

All patients had a high level of oral hygiene (full-mouth plaque index, $<10\%$) and their periodontal status was re-evaluated 8 weeks after initial treatment. The patients were scheduled for periodontal surgery if probing pocket depth (PPD) $\geqslant 5$ mm existed. The mean number of surgically treated teeth was four (minimum 2.5/maximum six). Each subject gave written informed consent regarding the study and was randomly assigned to the test, procedure or control procedure. A randomization list and randomization envelops were prepared by means of a random numbers table. The randomization procedure was not stratified for smoking status.

Surgical procedure

In each patient, one modified Widman flap was performed and full mucoperiostal buccal and lingual access flaps were raised. Granulation tissue and pocket epithelium were removed. Any remaining subgingival plaque and calculus were removed by scaling and root planing using manual scaler- and ultrasonic scaler (EMS S.A., Nyon, Switzerland). Minimal bone recontouring was performed, if deemed necessary by the clinician, to improve flap adaptation or to provide a better access for oral hygiene procedures after replacing the flap. A chisel or a bur cooled with sterile saline was used for bone recontouring. The surgical area was rinsed with saline and the mucoperiosteal flap was replaced. A continuous suture was made with silk suturing material (3/0 Ethicon Perma-Hand®, Johnson & Johnson Medical BV, Amersfoort, the Netherlands).

The suture was tightened, but no knot was placed. At this stage, the distance from the gingival margin to the alveolar bone and the distances from the cemento-enamal junction (CEJ) to the alveolar bone were measured, using a periodontal probe.

Next, in the control subjects the final knots were placed. In the test subjects the suture was carefully loosened, the wound area was irrigated with saline and enamel matrix proteins (EMD, Emdogain®, (Biora, 0.7 ml) were applied under the mucoperiostal flaps and onto the exposed root surfaces using a syringe with a short blunt-ended needle. When applying the enamel proteins, efforts were made to avoid contamination with saliva or blood of the surgical area. Subsequently, the suture was tightened and a final knot was placed.

The time used for surgery varied between 75 and 105 min.

Clinical measurements

Clinical measurements were taken at four time points. Table 1 shows which parameters were assessed during surgery and at 1, 4 and 8 weeks after surgery.

As clinical features of periodontal wound healing the following parameters were used:

1 swelling of the soft tissues,
2 color of the gingiva,

Table 1. Flow chart

Parameters: point of time (weeks)	PPD	CAL	PI	BI	Swelling	Color
0	X*,†	X*,‡	–	–	–	–
1	–	–	X	–	X	X
4	X	X	X	X	X	X
8	X	X	X	X	X	X

X, measurement; –, no measurement; PPD, probing pocket depth; CAL, clinical attachment level; PI, plague index; BI, bleeding index; CEJ; cemento-enamel junction.
*Immediately after suturing, but before knotting.
†Bone level-gingival margin.
‡Bone level-CEJ.

3 PPD,
4 CAL – clinical attachment level,
5 BI – bleeding index,
6 PI – plaque index.

Swelling of the gingiva was scored as follows: 0 = no swelling, 1 = moderate swelling, 2 = pronounced swelling. The color of the gingiva was recorded as follows: 0 = no redness, 1 = moderate redness, 2 = pronounced redness. The contra-lateral gingiva was used as a reference to judge changes in tissue color. PPD and CAL were recorded using a force-controlled probe (Brodontic® 0.75 N/cm^2, Ash/Dentsply, UK) with reference to the CEJ. Measurements were rounded off to the nearest millimeter. Plaque and bleeding were assessed for the area that had been operated and scored at six sites per tooth (or at the appropriate number of sites that are only partially involved in the flap procedure). Presence or absence of bleeding was recorded on a two point scale (no, or within 30 s after probing) while measuring the probing pocket depth and probing attachment levels at all examination intervals. Plaque was scored on a two-point scale, as absence or presence of plaque along the marginal gingiva.

All measurements were performed by the same examiner (S.H.), who was blinded for the treatments. Individual probes were assigned to each patient throughout the study in order to minimize variability (Van der Zee et al. 1991).

Seven days after surgery, the suture was removed and only visible signs of wound healing were measured. After four and 8 weeks, the swelling and the color of the gingiva, PPD, CAL, BI and PI were recorded.

Post-surgical hygiene and maintenance

To accomplish an effective level of plaque control in the surgical area, all the patients were instructed after surgery to rinse twice daily with 10 cm^3 of 0.2% chlorhexidin digluconate solution (Corsodyl®, SKB, Zeist, the Netherlands) for 1 min (Ernst 1998). Mechanical oral hygiene of the operated area was not allowed during the first week. Following removal of the sutures (after 7 days) patients were instructed to re-establish their manual oral hygiene procedures after 12–14 days post-operatively and to continue rinsing with chlorhexidin until 14 days post operation.

Post-surgical discomfort and adverse reaction

During the 7 days following surgery, patients filled out a questionnaire to evaluate the experience of post-operative complaints. Questions relating to experienced pain, sort of pain, use of analgesics, swelling and coloration of the face and swelling and bleeding of the gingival area were filled in by the patients. For the recording of the experienced pain, swelling of the operated area and swelling and coloration of the face, a Visual Analogue Scale (VAS score) was used. Patients were instructed not to return to former recordings while filling out each daily questionnaire.

Data analysis

Data were analyzed with the patient as the statistical unit. Mean values per patient were calculated for all clinical parameters. Furthermore, with respect to PPD and CAL, also for each assessment a mean was calculated for sites with baseline PPD $\geqslant$3 mm. Mann–Whitney tests were used to compare data at each assessment. Data considering the VAS scores of the questionnaire were analyzed using Mann–Whitney tests. p-Values $\leqslant$0.05 were considered as statistically significant.

Results

Patients

For this study, 22 patients volunteered to participate (nine female and 13 male) and were randomly assigned to test and control groups. Into the test group six women and five men were entered and into the control group three women and eight men. The mean age of all patients was 49.9 years (range, 32–67). In the test group, the number of operated teeth was 3.5 ($\pm$ 1.1, range, 3–6) and in the control group 4.0 ($\pm$ 0.9; range, 3 to 5), resulting in a mean of 21.0 operated sites in the test group and 24.0 sites in the control group.

By chance the randomization procedure resulted in an unequal distribution of the smokers over the control and test groups (0 versus 6). However, explorative testing for effects of this unequal distribution by comparing the smokers and non-smokers in the test group did not show any statistically significant differences for any of the clinical parameters at any assessment. Excluding the six smokers from the present analysis did not change the final results.

Clinical parameters

Table 2 shows a summary of the clinical data. At the time of surgery, the distance from the CEJ to the bone level was 5.18 for the test group and 5.22 for the control group. After suturing, the distance from the bone to marginal gingiva was 2.26 and 2.36 mm for the test and the control group, respectively. The ''initial pocket depth'' immediately following surgery remained unchanged up to 8 weeks post surgery. As a result of healing the gain in clinical attachment level was 0.42 and 0.67 mm in the two groups at the 8-week examination. For the control group this was a significant improvement from time of surgery. However, both for CAL and for PPD, no differences between groups were observed at any point in time.

In Table 3, a selection is made for ''pockets'' $\geqslant$3 mm at baseline examination. In the test group, the mean number of these sites per patient was 7.1 ($\pm$ 2.9) and in the control group this was 7.8 ($\pm$ 3.4).

At the time of surgery, the distance from the bone to marginal gingiva was on average 3.13 and 3.22 mm for the test and the control group respectively. The distance from the CEJ to the bone level was 5.85 and 5.68 mm. At the 8-week examination the reduction in PPD was 0.71 and 0.74 mm, respectively, which was a significant change from baseline in both groups. As a result of healing the gain in CAL was 0.81 and 0.95 mm, respectively. In these deeper post-surgical pockets ($>$3 mm) also no statistically significant differences between groups were observed.

The mean bleeding index at the 8-week examination ranged between 0.16 and 0.18 and was not different between groups.

The mean plaque index (see Table 2) was 0.14 and 0.13 for test- and control groups at the first week during which time patients had been rinsing with chlorhexidin.

After they had resumed brushing and interdental cleaning at the 8-week appointment it was 0.21 and 0.22, respectively.

Table 2. Mean clinical indices with standard deviation in parentheses

Variable	Surgery*		Week 1†		Week 4		Week 8	
	exp.	contr.	exp.	contr.	exp.	contr.	exp.	contr.
swelling	–	–	0.48(0.31)	0.26(0.43)‡	0.26(0.45)	0.08(0.13)	0.02(0.06)	0.05(0.15)
redness	–	–	0.80(0.44)	0.80(0.50)	0.48(0.56)	0.40(0.39)	0.17(0.21)	0.16(0.33)
PPD	2.26(0.34)	2.36(0.42)	–	–	2.37(0.69)	2.14(0.39)	2.23(0.38)	2.17(0.42)
CAL	5.18(0.66)	5.22(0.80)	–	–	4.86(0.95)	4.74(0.70)	4.76(0.90)	4.55(0.81)§
bleeding	–	–	–	–	0.30(0.21)	0.18(0.18)	0.16(0.16)	0.18(0.23)
plaque	–	–	0.14(0.18)	0.13(0.15)	0.18(0.20)	0.19(0.22)	0.21(0.16)	0.22(0.21)

*The tip of the probe was placed at the bone level.
†No subgingival probing at 1 week post-operative, therefore only supra-gingival measurements are given.
‡Statistical significant difference between groups, $p<0.05$.
§Statistical significant difference from the time of surgery, $p<0.05$.
exp. = Experimental; contr. = control; PPD = probing pocket depth; CAL = clinical attachment level.

Table 3. Mean clinical indices for pockets $\geqslant 3$ mm at baseline examination, with standard deviation in parenthesis

	Surgery*		Week 4		Week 8		*N* (sites)†	
	exp.	contr.	exp.	contr.	exp.	contr.	exp.	contr.
PPD	3.13(0.14)	3.22(0.27)	2.62(0.89)‡	2.53(0.49)‡	2.42(0.41)‡	2.48(0.44)‡	7.1(2.9)	7.8(3.4)
CAL	5.85(0.81)	5.68(1.12)	5.24(1.30)	4.87(1.27)‡	5.04(1.13)	4.73(1.06)‡	7.1(2.9)	7.8(3.4)

*The tip of the probe was placed at the bone –level.
†Mean number of sites with baseline PPD $\geqslant 3$ mm per patient.
‡Statistical significant difference from the time of surgery, $p<0.05$.
exp. = Experimental; contr. = control; PPD = probing pocket depth; CAL = clinical attachment level.

Table 4. Individual data regarding the swelling score of the gingiva 1 week post-operative

	Swelling	
	exp.	contr.
	0.15	0
	0.21	0
	0.22	0.03
	0.33	0.04
	0.39	0.06
	0.39	0.11
	0.50	0.11
	0.56	0.13
	0.63	0.13
	0.67	1.10
	1.28	1.17
Mean (SD)	0.48 (0.31)	0.26 (0.43)

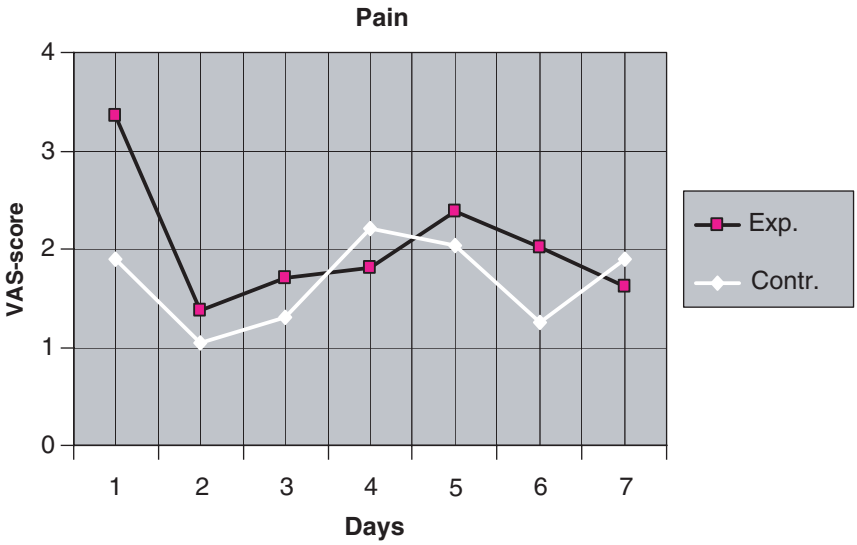

Fig. 1. Results of questionnaire: How much pain did you experience? On a scale from ''no'' pain to ''very much'' pain.

Swelling and redness showed no significant differences between groups at any of the time points, except for gingival swelling at week 1. At this time point, the mean swelling score was 0.48 and 0.26 for the test and the control group, respectively. This difference was statistically significant ($p<0.05$). Individual data are presented in Table 4 and they clearly indicate the origin of those differences.

Questionnaire

Figs 1–4 and Table 5 show a summary of the data from the questionnaires.

Evaluating the VAS-scores of the questionnaires no significant differences were observed between both groups. On a scale from 0 to 10, the pain experienced at day 1 was 3.35 for the test and 1.90 for the control group (Fig. 1). So, both were on the low end of the scale. This decreased to 1.61 in the test group

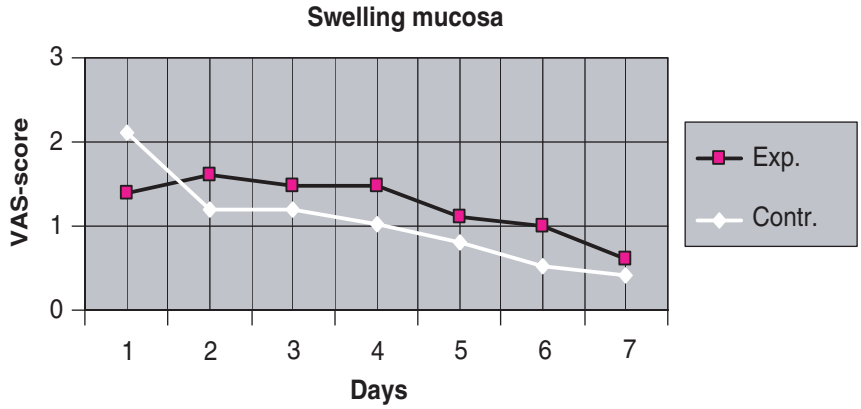

Fig. 2. Results of questionnaire: How much swelling of the operated area did you experience? On a scale from ''no'' swelling to ''very much'' swelling.

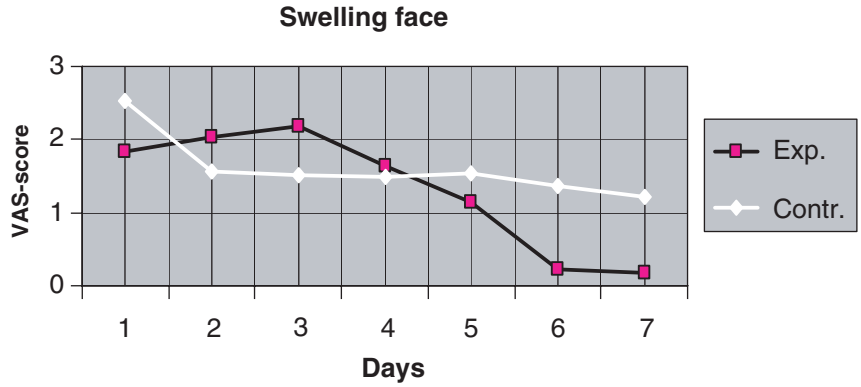

Fig. 3. Results of questionnaire: How much swelling of the face did you experience? On a scale from ''no'' swelling to ''very much'' swelling.

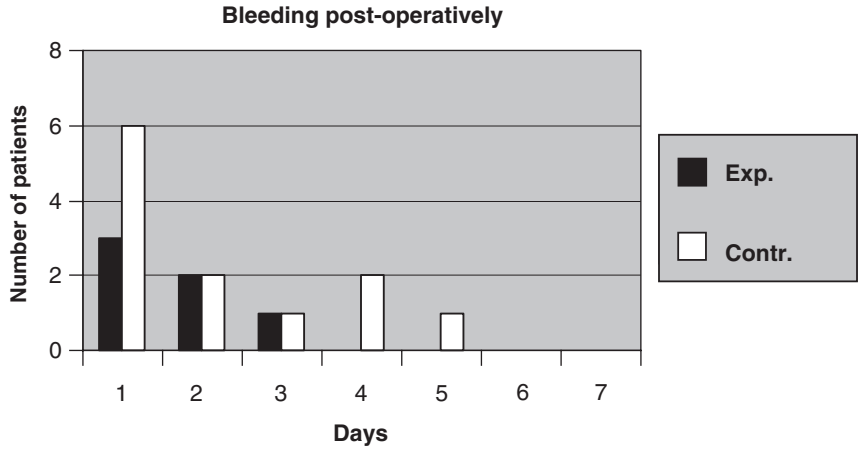

Fig. 4. Results of questionnaire: Does the operated area bleed, yes/no.

and remained 1.90 in the control group at day 7.

Swelling of the mucosa, swelling of the face (Figs 2 and 3) was observed in both groups.

Descriptive analysis of the data indicates that the swelling and discoloration of the face was reported as most pronounced at the first day for the control group (VAS scores of 2.54 and 1.07, respectively) and the third day after surgery for the test group (VAS scores of 2.17 and 1.05, respectively). The swelling of the face (Fig. 3) almost disappeared in the test group 0.18 (day 7) whereas in the control group some swelling of the face was still experienced 1.22 (day 7). No statistically significant differences were found for these parameters.

Table 5 shows the mean number of analgetics used in each group per day.

The mean number used during the first day in the test group and control group was 2.27 and 1.73, respectively. After the first day, the mean intake of analgetics decreased to 0.91 and 1.09 at day 7.

Fig. 4 shows the reports of post-operative oozing of the wound.

The first day after surgery, twice as many patients experienced oozing of the wound in the control group as in the test group. Oozing of blood from the wound area was still present until the fifth day in the control group, while in the test group this disappeared at the third day.

Discussion

The intent of the current study was not to evaluate per se the regenerative potential of EMD. Rather the soft-tissue wound healing was monitored by clinical methods. It has been suggested that the hydrophobic properties of EMD might interact with the colonization of the tooth surface by oral microorganisms. If EMD influences selective attachment and outgrowth of oral bacteria, preferably those present in a healthy periodontium, and suppresses microorganisms associated with periodontitis, this protein mixture might help in regaining a healthy periodontium (van der Pauw et al. 2000, 2002)

In vitro studies have reported antimicrobial effects of EMD. Freshly prepared Emdogain® has an inhibitory effect on the growth of three periodontal pathogens, *Actinobacillus actinomycetemcomitans*. *Porphyromonas gingivalis* and *Pre intermedia*. A similar effect was found for the vehicle propylene glycol alginate (PGA) alone in which EMD is normally dissolved. Spahr et al. (2002) suggested that the growth inhibition can be mainly attributed to the vehicle PGA. This might be the result of the low pH between 4 and 5 and has been confirmed by Arweiler et al. (2002). Using ex vivo plaque samples,

Table 5. Mean number of analgesic tablets taken in each group with standard deviation in parentheses

Day	1	2	3	4	5	6	7	Total
exp.	2.27(1.35)	1.09(1.58)	1.27(1.74)	1.36(1.80)	1.45(1.63)	1.00(1.67)	0.91(1.70)	9.36(10.29)
contr.	1.73(0.90)	1.36(1.43)	1.27(1.56)	1.36(1.75)	1.18(1.47)	1.09(1.38)	1.09(1.45)	9.09(8.99)

exp., experimental; contr., control.

Sculean et al. (2001) observed that EMD had an antibacterial effect on the vitality of supragingival dental plaque flora. This antibacterial effect could contribute to its beneficial effects of EMD on wound healing.

It was the aim of this study to evaluate the effect of EMD application on the initial wound healing after flap surgery. Although it is generally recommended to wait for 6 months before taking any clinical measurements after enamel matrix proteins are applied, this study used the clinical assessments not to evaluate the regenerative potential but as measures of healing of gingival tissues which have been deflected from the bone and tooth surface during periodontal surgery. The results show that no clinical difference could be established between surgery with or without EMD application with respect to the clinical outcome parameters as assessed in this study.

This outcome is in agreement with the outcome of a study from Zetterstrom et al. (1997), who found no differences in adverse post-surgical experiences between test and control groups. It supports the conclusion that EMD is a clinically safe product which does not have a negative impact on wound healing (Heard et al. 2000). Table 2 shows that the gain in attachment 1 month after surgery is 0.32 mm in the test group and 0.48 mm in the control group. If sites which measure $\geqslant 3$ mm at baseline are selected, this gain is 0.61 and 0.81 mm, respectively. Pontoriero & Carnevale (2001) assessed the alterations of the marginal periodontal tissues as an immediate outcome of surgical crown lengthening and over a 12-month healing period. They showed a mean gain in clinical attachment following surgical crown lengthening on the interproximal sites of 0.5 mm as measured 1 month after surgery.

By chance the randomization procedure resulted in an unequal distribution of the smokers over the control and test groups (0 versus 6). It is generally accepted that smoking has an adverse affect on wound healing. It slows down the healing and decreases the defense against bacteria (Barbour et al. 1997). However, excluding the six smokers from the present analysis did not change the results.

Following a periodontal surgical procedure a patient may experience some degree of swelling, pain, bleeding, and root sensitivity (Curtis et al. 1985) Approximately 50% of the patients undergoing a periodontal surgical procedure may experience mild post-operative pain, and 4.6% may experience severe pain. Swelling is a common finding seen in approximately 80% of the patients. The results of the present study show that although post-operative symptoms were present, the patient perception of these was considerately mild to little since the mean score was on the low end of the 10-point scale. This was the same for both groups.

In conclusion this study has shown that the initial wound healing of periodontal flap surgeries in those sites treated with Emdogain® is not different from the healing of control sites.

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